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Occurrence of *Trichograma* Parasitoids in Eggs of Soybean Lepidopteran Pests in Mato Grosso, Brazil

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Abstract

Occurrence of parasitoids in eggs of soybean lepidopteran pests, such as velvetbean caterpillar, soybean loopers and others the genus *Spodoptera* and *Helicoverpa* in Mato Grosso, Brazil was studied. Due to the scarcity of information on the natural occurrence of parasitoids in lepidopteran eggs in soybean fields in Mato Grosso surveys were conducted in Tangará da Serra to verify the occurrence these parasitoids. Surveys were conducted in two areas in Tangará da Serra, with a total of 591 eggs, of which 138 and 77 eggs were collected in area I and 274 and 102 eggs in area II, during the 2008/2009 and 2009/2010 soybean seasons, respectively. From the eggs collected in area I, 26.6% were parasitized in the first year and 30.0% in the second season. In area II, the parasitism rates were 40.2% and 27.5% for the first and second season respectively. The species of parasitoid collected was *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) totaling 354 parasitoids. We conclude that there is a natural occurrence of *T. pretiosum*, this being the species of egg parasitoid found, and that parasitism rates were higher when compared with other studies under natural conditions, especially considering the large number of chemical products used in the fields. The identification of the eggs parasitoids in Mato Grosso State is important to development of biological control programs for lepidopteran pests.

Keywords: insecta, natural enemies, Noctuidae, biocontrol, pesticides, Brazil

1. Introdution

Brazil is a major producer of soybeans (*Glycine max* L.), and the state of Mato Grosso is the major producer of this grain, with a planted area of 6.16 million hectares, accounting for 27.5% of the national production (CONAB, 2010). However, the large size of the area occupied by soybeans favors the incidence of insect pests in the crop (Corrêa-Ferreira & Panizzi, 1980). Among the pests that attack soybeans defoliating caterpillars, such as velvetbean caterpillar, soybean loopers and others the genus *Spodoptera* and *Helicoverpa* stand out for decreasing leaf area, and consequently lowering the photosynthetic rate which decreases productivity, while leaving the plant susceptible to disease and sometimes causing its death. In most cases these pests are controlled with wide spectrum chemicals that reduce the action of natural enemies. However, the pests eventually become resistant, while the chemicals pose health hazards and environmental contamination.

For these reasons, states such as Rio Grande do Sul (Costa & Link, 1974), Paraná (Corrêa-Ferreira et al., 1998; Foerster & Avanci, 1999), São Paulo (Campos et al., 1997), Mato Grosso do Sul (Godoy et al., 2005), Minas Gerais (Venzon et al., 2000), Distrito Federal (Medeiros et al., 1997) and Acre (Thomazini, 2001) have developed research on species of insect pests and natural enemies, aiming at the implementation of biological control programs.

Among the natural enemies that act in reducing pest populations, egg parasitoids are very effective, since they prevent the development of the pest while still in the egg stage and thus avoid damage to the crop. Egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are important because they parasitize eggs of Lepidoptera, Hemiptera and Coleoptera that attack various crops (Parra & Zucchi, 2004) and, for this reason, are used in biological control programs worldwide (Beserra & Parra, 2004). Currently, there are 190 known species of *Trichogramma*, and 38 of these occur in South America, 28 of which have been recorded in Brazil (Moreira et al., 2009). Despite the importance of soybean production in the state of Mato Grosso, no research has been carried out in the state regarding the occurrence of egg parasitoids, although their effectiveness has been proven by previous research (Foerster & Avanci, 1999; Foerster & Butnariu, 2004).

Due to the scarcity of information on the natural occurrence of parasitoids in lepidopteran eggs in soybean fields in Mato Grosso, surveys were conducted in two areas in Tangará da Serra with the aim to verify the occurrence of the *Trichogramma* parasitoids and assess the rates of parasitism. The occurrence of the phytophagous Lepidoptera was also assessed, as well as the pesticides used in the study areas.

2. Materials and Methods

2.1 Study Areas

The study was conducted in two areas located in the municipality of Tangará da Serra, Mato Grosso, Brazil, cultivated with soybean. The first area (area I) is cultivated with conventional soybean and a dense submontane forest reserve (14°39′53″S and 57°24′30″W). The second area (area II), was planted with organic soybean until the previous year and then conventionally during the sampling season. This area has Cerrado reserves (14°18′44″S and 57°45′18″W) and is located 90 km away from area I. Temperature and relative humidity (RH) of the areas were obtained using a digital thermohygrometer on the sampling days. The average temperature and RH were 28.6 ºC and 59.8% in area I and 26.8 ºC and 67.5% in area II.

2.2 Sampling of Lepidopteran Eggs

Eggs of Lepidoptera were collected during the crop seasons of 2008/2009 and 2009/2010 in the months of November, December and January (vegetative period and early reproductive period). Two samplings were performed per month for 1.5 hours each, except for the second crop in the area II, where monthly samples were collected.

The sampling methodology used was described by Foerster and Avanci (1999): soybean leaves containing eggs were collected and taken to the laboratory where the eggs were removed from the leaves with the help of a fine brush under a stereomicroscope and individualized in 1.5 ml microtubes which were kept in conditions controlled of BOD at 25 °C and 70% relative humidity.

The occurrence of parasitism was checked daily and the number of emerged parasitoids per host egg and sex of the individuals determined by the morphological characteristics of the antennae (Querino & Zucchi, 2003). When parasitoids emerged, females were placed in microtubes containing 70% ethanol and males were used to identify the species.

2.3 Identification of Parasitoids

Specimens were identified according to the morphological characters of the male genitalia (Nagaraja & Nagarkatti, 1973). Parasitoids were cleared in acetic acid for 24 hours, and mounted individually on glass slides in a micro-droplet of Hoyer's medium. Specimens were mounted with their ventral side up and legs pushed to the side, leaving the genitals free for observation. Another micro-droplet was placed on the parasitoid followed by the coverslip. After confirming the sex, the slides were sent for identification to Dr. Ranyse B. Querino of EMBRAPA Meio-Norte.

Identified specimens were deposited in the entomological collection of the laboratory of Zoology at the Centro de Pesquisas, Estudos e Desenvolvimento Agro-ambientais (CPEDA) at the Universidade do Estado de Mato Grosso campus de Tangará da Serra (UNEMAT/CUTS).

2.4 Sampling of Lepidopteran Larvae

All larvae found at the sampling points were collected manually in order to record the lepidopteran species found in the area. These were taken to the laboratory for identification using books and field guides. The identification of adults by an expert was not possible, since chemical products that were applied in the areas prevented the collected larvae from reaching the adult stage.

2.5 Pesticides Used

Using a predefined form, an interview was conducted with the persons responsible for applying the pesticides in the fields to obtain the names of the products used.

In both areas studied chemical management is the only method of pest control used. The main insecticides used were Lambda-cyhalothrin (Karate Zeon®), Thiodicarb (Larvin®), Thiomethoxam (Engeo Pleno®), Metal phosphides (Fosfito®), Organophosphates (Metamidofós®). The herbicides used were Chlorimuron (Classic®), Fomesafen (Flex®), Fluazifop (Fusilade®), Glyphosate (Roundup®), and the fungicides were Azoxystrobin and Cyproconazole (Priori Xtra®) and Triactol (Alto 100®). All pesticides used were recommended by agronomists.

2.6 Data Analysis

We calculated the mean percentage of parasitism in the study areas and analyzed the data using a one way analysis of variance ($p \leq 0.05$) (ANOVA). The sex ratio was calculated for the parasitoids collected in the 2009/2010 season, using the formula: rs = №♀ / (№♀ + №♂). The average number of parasitoids emerged per egg was also calculated.

3. Results and Discussion

A total of 591 eggs were collected in the two areas, of which 138 and 77 eggs were collected in area I and 274 and 102 eggs area II, during the 2008/2009 and 2009/2010 soybean seasons, respectively. From the eggs collected in area I, 26.6% were parasitized in the first year and 30.0% in the second season. In area II, the parasitism rates were 40.2% and 27.5% for the first and second season, respectively (Figure 1). The average parasitism in two areas was 35.7%, without significant difference between the two areas ($F = 0.49$ and $P = 0.68$ ($p \leq 0.05$).

Despite the fact that the parasitoids were influenced by the host and the chemical management employed in the areas (Zago et al., 2010), percentages of parasitism in Mato Grosso were higher than those in Paraná, where Avanci et al. (2005) found that parasitism in eggs of *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae) varied from 3.7% to 11.5% during four seasons.

The species of parasitoid collected was *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) totaling 354 parasitoids. According to Gonçalves et al. (2003), this species is the most widely distributed in Brazil and, according to Zucchi and Monteiro (1997), it is the most generalist and is associated with 26 host species (Potrich et al., 2009).

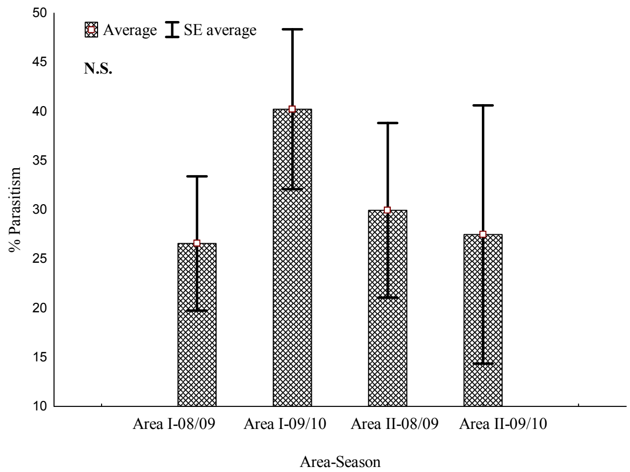

Figure 1. Percentage of eggs of noctuid pests on soybean parasitized by *Trichograma pretiosum*. The two areas were sampled in Mato Grosso, Brazil during the 2008/2009 and 2009/2010 growing seasons. The rates of parasitism were not significantly different according to ANOVA ($F = 0.49$; $P = 0.68$; $p \leq 0.05$)

Avanci et al. (2005) also recorded a predominance of *T. pretiosum*, which accounted for 80% of the parasitized eggs of *A. gemmatalis*. In another study in Paraná, during five consecutive soybean seasons Foerster and Avanci, (1999) recorded 90% of the eggs of *A. gemmatalis* parasitized by *T. pretiosum*. In maize (*Zea mays* L.) crops in Minas Gerais, Tironi and Ciociola (1994) recorded more than 90% of the eggs of *Helicoverpa zea* (Boddie, 1850) (Lepidoptera: Noctuidae) parasitized by *T. pretiosum*.

According to Moreira et al. (2009), in Brazil, *T. pretiosum* is the most frequently occuring parasitoid and is associated with various hosts in different cultures, such as *Tuta absoluta* (Meyrick, 1917) in tomato (Pratissoli et al., 2005), *Spodoptera frugiperda* (Smith, 1797) (Bessera & Parra, 2003) and *H. zea* (Pratissoli & Oliveira, 1999) in maize, *Plutella xylostella* (Linnaeus, 1758) in cabbage (Pereira et al., 2004; Zago et al., 2010), *Heliothis virescens* (Fabricius, 1781) in cotton (Zucchi et al., 1989), *A. gemmatalis* and *Chrysodeixis* (= *Pseudoplusia*) *includens* (Walker, 1858) in soybean (Bueno et al., 2009).

Three species of phytophagous Lepidoptera were found in area I, during two seasons, of which 74.9% was *C.*

includens, 20.5% *A. gemmatalis* and 4.6% *Spodoptera* spp. In area II there was an inversion of these data, being 69.4% *Spodoptera* spp., 19.2% *A. gemmatalis* and 11.4% *C. includens*.

This inversion can be related to the type of management used as in area I, where regular insecticide applications were made, since the area has always been planted with conventional soybean. This may have favored the emergence of resistant strains of *C. includens*, justifying the large number of this species. The high number of *Spodoptera* spp. Can be related to the conventional maize has been cultived after last organic soybean cultivation. Maize cultivastion was not appropriately carried out, leaving residues on the crop.

Considering the presence of larvae, we concluded that the parasitized eggs belong to these species since the identification of eggs is difficult due to the darkening of the egg as the parasitoid develops inside the eggs. Thus, all eggs were included in the count. However, the method of oviposition found in the areas (individual eggs on leaves) which is characteristic of *A. gemmatalis* and *C. includens*, and leads us to infer that these species were predominant when compared to eggs of *Spodoptera* spp., which are laid in masses of more than 100 eggs.

The sex ratio is an important feature of biological control programs, since the greater the production of females, the greater the number of parasitized eggs and parasitoids in the next generation. In this study, the sex ratio of the parasitoids was 0.5 ± 0.10 for eggs collected in area I and 0.6 ± 0.07 for eggs collected in area II during the 2009/2010 season. Avanci et al. (2005) obtained similar results in eggs of *A. gemmatalis* under natural conditions as the ones recorded in the area II (0.65 ± 0.02) for *T. pretiosum*. Bueno et al. (2009) observed, under laboratory conditions, similar results in eggs of *C. includens* (0.70 ± 0.02). Thus we can infer that environmental differences do not affect the sex ratio of the parasitoids.

Of the eggs collected, the average number of adults emerged per egg was (average ± SE) 1.4 ± 0.46 and 1.6 ± 0.36 in I area (area with predominance of larvae of *C. includens*) and 1.6 ± 0, 11 and 1.3 ± 0.06 in II area (where occurrence of *A. gemmatalis* was higher than that of *C. includens*) in the 2008/2009 and 2009/2010 seasons, respectively. Bueno et al. (2009) obtained lower emergence rates for *C. includens* (1.0 ± 0.0) whereas Avanci et al. (2005) recorded higher emergence of parasitoids in eggs of *A. gemmatalis* (2.2 ± 0.06).

Differences in the number of adults emerged per egg can be associated with the host species. Nutritional characteristics, egg size, and adaptations of the parasitoid are very important in this process (Bueno et al., 2009).

As explained above, pesticides were utilized during sampling periods, and the use of chemicals also influenced the viability of parasitoids. Bueno et al. (2008) showed a 50% reduction in the viability of eggs parasitized by *T. pretiosum* in laboratory with the use of products such as Lambda-cyhalothrin and Thiodicarb. Moreover, other products such as glyphosate and chlorimuron reduced by 100% the viability of parasitized eggs in laboratory tests, and all these products were used in the areas sampled in our study.

Despite the use of these products in the study areas, parasitoids were present throughout the period the collections were made. Figures 2A and 2B show the percentage of parasitism for each sampling.

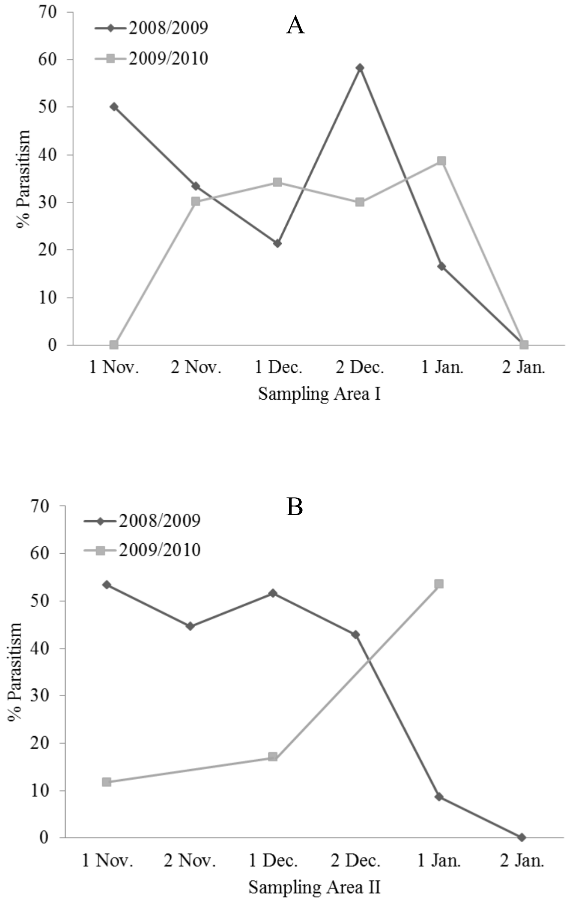

Figure 2. Population fluctuation of *Trichogramma pretiosum* egg parasitoids during the 2008/2009 and 2009/2010 soybean seasons in the two areas sampled in Mato Grosso, Brazil

During the first year, in area I, the parasitoids were present from the first sampling (Figure 2A), and the level of parasitism was higher when compared to the second season. Declines in the percentage of parasitism, as seen in the samplings made in November (2 Nov.) and December (1 Dec.), may be related to the use of pesticides, increasing mortality of caterpillars and unavailability of eggs.

In area II (Figure 2B), during the first season, since the beginning of tsampling, parasitism remained similar and, in January, when soybeans entered the stage of pre-harvest, there was a decrease in the percentage of parasitism. In the second soybean season, due to difficulties in accessing the area, there were only three samples taken., They showed inverse relation to the first year, which may also be related to the use of chemical products as this was the second year of chemical use in the area.

4. Conclusions

Concluded that *Trichogramma pretiosum* was the only species parasitizing lepidopteran eggs on soybeans in our sampling areas, and that the parasitism rate was higher than recorded in other Brazilian regions. The lepidopteran pests the most abundant lepidopteran species in the areas investigated are *Chrysodeixis includes*, *Anticarsia gemmatalis* and *Spodoptera* sp.

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